**RESEARCH ARTICLE**

**Lyplal1** is dispensable for normal fat deposition in mice

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**ABSTRACT**

Genome-wide association studies (GWAS) have detected association between variants in or near the Lysophospholipase-like 1 (LYPLAL1) locus and metabolic traits, including central obesity, fatty liver and waist-to-hip ratio. **LYPLAL1** is also known to be upregulated in the adipose tissue of obese patients. However, the physiological role of **LYPLAL1** is not understood. To investigate the function of **Lyplal1** in vivo we investigated the phenotype of the **Lyplal1**<sup>tm1a(KOMP)Wtsi</sup> homozygous mouse. Body composition was unaltered in **Lyplal1** knockout mice as assessed by dual-energy X-ray absorptiometry (DEXA) scanning, both on normal chow and on a high-fat diet. Adipose tissue distribution between visceral and subcutaneous fat depots was unaltered, with no change in adipocyte cell size. The response to both insulin and glucose dosing was normal in **Lyplal1**<sup>tm1a(KOMP)Wtsi</sup> homozygous mice, with normal fasting blood glucose concentrations. RNAseq analysis of liver, muscle and adipose tissue confirmed that **Lyplal1** expression was ablated with minimal additional changes in gene expression. These results suggest that **Lyplal1** is dispensable for normal mouse metabolic physiology and that despite having been maintained through evolution **Lyplal1** is not an essential gene, suggesting possible functional redundancy. Further studies will be required to clarify its physiological role.

**KEY WORDS: Adipose tissue, Genome-wide association study, Lyplal1, Model organism, Mouse, Obesity**

**INTRODUCTION**

Lysophospholipase-like 1 (LYPLAL1) is a protein with a poorly understood biological role, despite its evolutionary conservation (Fig. S1). The crystal structure of **LYPLAL1** is similar to that of APT1 (acyl protein thioesterase 1, also known as **LYPLAL1**), but the shape of its active site indicates that unlike APT1, which depalmitoylates Gα and Rα proteins, it cannot bind long-chain substrates. Biochemical data confirm this, also demonstrating that **LYPLAL1** accepts short-chain 4-nitrophenyl esters (Bürger et al., 2012). Despite identification of a small-molecule inhibitor, the natural substrate of **LYPLAL1** and its physiological role remain unknown.

**RESULTS**

**Verification of **Lyplal1** knockout**

Mice with loss of **Lyplal1** (termed **Lyplal1**<sup>tm1a/tm1a</sup>) were generated using the tm1a knockout first allele design as part of the International Mouse Phenotyping Consortium (IMPC) project (Fig. 1A). **Lyplal1** mRNA levels were negligible in all organs tested, with the tm1a allele resulting in >95% knockout of **Lyplal1** at the RNA level in the kidney and gastrocnemius muscle, and >99% knockout in all other tissues tested (heart, liver, spleen and adipose tissue; Fig. 1B-I). Furthermore, RNAseq confirmed loss of **Lyplal1** expression, consistent with the gene construct, with only a few detectable reads mapping to exon 1 (Fig. S2). No other exons display complete coverage in any of the **Lyplal1**<sup>tm1a/tm1a</sup> samples investigated, with no more than three reads at any one base outside exon 1. The reduction in reads for exon 1 might be attributable to disruption of an unmapped regulatory element, such as an enhancer in the first exon, or a result of nonsense-mediated decay. The allele
design is such that transcription is prevented beyond the lacZ in the inserted cassette. However, in the case of any skipped splicing over the cassette, the resulting transcripts would be frameshifted and subject to nonsense-mediated decay. It might also be possible that the Exon1::LacZ transcript is detected as aberrant and degraded.

Lyplal1 was undetectable in all protein samples collected from homozygous Lyplal1 tm1a/tm1a mice (Fig. 1J; Fig. S3).

**Body composition**

No obvious body weight or metabolic phenotype was observed for Lyplal1 tm1a/tm1a mice during standardized phenotyping (www.mousephenotype.org/data/genes/MGI:2385115) (White et al., 2013). To investigate further the role of Lyplal1 on adipose tissue development, and other relevant metabolic phenotypes, mice were challenged with a HFD from 6 weeks of age. Body weight and nose-to-tailbase length were not altered in HFD-fed Lyplal1 tm1a/tm1a mice, compared with wild type (Fig. 2A; Fig. S4A,B). Lean and fat mass composition and bone mineral parameters were also unaltered in Lyplal1 tm1a/tm1a mice at 14 and 24 weeks of age (Fig. 2B-D; Fig. S4C-K). To investigate fat distribution, vWAT, scWAT and brown adipose tissue were dissected from 28-week-old mice and weighed. No change was detected in Lyplal1 tm1a/tm1a mice (Fig. 2E-G), indicating that
**Lypla1** is dispensable for adipose tissue distribution and size in mice. All other organ weights measured (liver, kidney, gastrocnemius and tibialis anterior muscles, heart and spleen) were similar to wild type in **Lypla1** tm1a/tm1a mice at 28 weeks of age (Table S1).

To determine whether adipose tissue architecture was altered, adipocyte cross-sectional area (CSA) was determined from scWAT sections (Fig. 2H–I). No changes were observed, either in average adipocyte CSA (Fig. 2I) or in the distribution of cell.
CSA (Fig. S5). Furthermore, liver cryosections showed no qualitative differences in Lyplal1tm1a/tm1a mice compared with wild type (Fig. 2J), indicating that knockout of Lyplal1 does not alter adipocyte size or the extent of fatty liver in adult mice on a HFD.

Concentrations of plasma lipids (cholesterol, high-density lipoprotein, low-density lipoprotein, non-esterified fatty acids and triglycerides) and other metabolically relevant parameters (albumin, alkaline phosphatase, alanine transaminase, amylase, aspartate aminotransferase, creatine kinase, creatine, fructosamine and glycerol) were determined in 28-week-old mice after a 4 h fast (Table 1). No parameters were altered in Lyplal1tm1a/tm1a mice compared with wild type, again suggesting that Lyplal1 does not influence the regulation of these parameters.

**Glucose homeostasis**

Owing to potential links with fasting insulin concentration, insulin clearance and insulin resistance, we investigated glucose homeostasis in Lyplal1tm1a/tm1a mice. Fasting blood glucose was measured at three ages after varying durations of fast. Although concentrations differed according to sex, Lyplal1 knockout did not alter fasting blood glucose in any conditions tested (Fig. S6). The responses to insulin and glucose doses were tested via intraperitoneal insulin and glucose tolerance tests at 18 and 22 weeks of age, respectively. Lyplal1tm1a/tm1a mice did not show an altered response to either challenge (Fig. 3A-D). Plasma insulin concentrations in 28-week-old Lyplal1tm1a/tm1a mice after a 4 h fast were also unaltered (Fig. 3E). Collectively, these results indicate that loss of Lyplal1 does not profoundly alter glucose homeostasis.

**Indirect calorimetry**

Indirect calorimetry was performed on 26-week-old mice for 48 h, to collect data on food intake, energy expenditure and activity levels. Activity levels and food intake during calorimetry were normal in Lyplal1tm1a/tm1a animals (Fig. 3F; Fig. S7A,B,G,H). Although there was a small significant difference in O2 uptake in Lyplal1tm1a/tm1a mice, the energy expenditure, CO2 output and respiratory exchange ratio remained unaltered, and traces through the time period were all qualitatively similar (Fig. 3G; Fig. S7C-F,J). Overall, the calorimetry did not demonstrate major changes in energy homeostasis attributable to Lyplal1 loss.

**RNaseq**

As no phenotype alterations were observed in Lyplal1tm1a/tm1a mice, we sought to investigate whether there were compensatory changes in gene expression that might explain the apparent redundancy in Lyplal1 function. We performed differential gene expression analysis of RNaseq data obtained from metabolically relevant tissues [liver, skeletal muscle (gastrocnemius), scWAT and vWAT]. As expected, Lyplal1 was the top differentially expressed gene between knockout and wild type for all tissues, with additional differentially expressed genes (P<0.05, adjusted for multiple testing) listed in Table 2. Lyplal1 was the only differentially regulated gene in gastrocnemius muscle, with only one additional gene differentially regulated in liver (Niuak1), three additional genes in scWAT and 11 additional genes in vWAT. Despite meeting the significance threshold, all these additional genes have low log-fold changes (0.2-0.75), indicating that knockout of Lyplal1 does not cause substantial changes in the transcriptome in mice.

**DISCUSSION**

LYPLAL1 has been linked to many metabolic phenotypes in humans and rodents, through GWAS and expression studies (Bandstein et al., 2016; Benjamin et al., 2011; Bille et al., 2011; Chu et al., 2017; Fox et al., 2012; Goodarzi et al., 2013; Heid et al., 2010; Lei et al., 2015; Lindgren et al., 2009; Manning et al., 2012; Randall et al., 2013; Schmid et al., 2012; Scott et al., 2012; Speliotes et al., 2011; Steinberg et al., 2007). However, these results are correlative and do not demonstrate direct causality of LYPLAL1 on associated phenotypes. We therefore aimed to investigate the in vivo role of Lyplal1 in metabolic regulation and adipose tissue deposition. We studied Lyplal1tm1a/tm1a mice obtained from the Knockout Mouse Project (KOMP) repository, with primary phenotyping obtained by the Sanger Institute Mouse Genetics Project (MGP) (White et al., 2013).

In our hands, Lyplal1tm1a/tm1a mice did not display a detectable metabolic phenotype, even under a HFD challenge. Loss of Lyplal1 expression was confirmed at both the RNA and the protein level, with RNaseq data showing few detectable reads mapping to exon 1, consistent with the gene construct. This suggests that the lack of obvious phenotype was not attributable to residual Lyplal1 expression. In the absence of a detectable phenotype, there was the possibility that another gene was compensating for Lyplal1. However, our RNaseq data demonstrate minimal expression.

### Table 1. Concentrations of the listed parameters in plasma collected from 28-week-old knockout mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td></td>
<td>Lyplal1+/+</td>
<td>Lyplal1tm1a/tm1a</td>
</tr>
<tr>
<td>Albumin (g l⁻¹)</td>
<td>27.07±1.575</td>
<td>26.89±1.508</td>
</tr>
<tr>
<td>ALP (U l⁻¹)</td>
<td>81.60±16.61</td>
<td>88.79±22.67</td>
</tr>
<tr>
<td>ALT (U l⁻¹)</td>
<td>213.9±103.7</td>
<td>223.6±110.6</td>
</tr>
<tr>
<td>Amylase (U l⁻¹)</td>
<td>571.8±44.44</td>
<td>577.4±62.48</td>
</tr>
<tr>
<td>AST (U l⁻¹)</td>
<td>218.9±67.96</td>
<td>202.1±88.21</td>
</tr>
<tr>
<td>Cholesterol (mmol l⁻¹)</td>
<td>5.411±0.861</td>
<td>5.404±0.823</td>
</tr>
<tr>
<td>CK (U l⁻¹)</td>
<td>359.1±162.8</td>
<td>290.1±194.0</td>
</tr>
<tr>
<td>Creatinine (µmol l⁻¹)</td>
<td>8.911±1.040</td>
<td>7.878±1.353</td>
</tr>
<tr>
<td>Fructosamine (µmol l⁻¹)</td>
<td>193.9±8.35</td>
<td>189.3±7.42</td>
</tr>
<tr>
<td>Glycerol (µmol l⁻¹)</td>
<td>199.4±28.32</td>
<td>204.7±32.66</td>
</tr>
<tr>
<td>HDL (mmol l⁻¹)</td>
<td>3.499±0.392</td>
<td>3.416±0.396</td>
</tr>
<tr>
<td>LDL (mmol l⁻¹)</td>
<td>1.122±0.288</td>
<td>1.130±0.224</td>
</tr>
<tr>
<td>NEFAC (µmol l⁻¹)</td>
<td>0.502±0.086</td>
<td>0.576±0.121</td>
</tr>
<tr>
<td>Triglycerides (mmol l⁻¹)</td>
<td>0.444±0.065</td>
<td>0.476±0.076</td>
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Blood was collected retro-orbitally after a 4 h fast and plasma analysed using an Olympus AU400. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFAC, non-esterified fatty acids (n=9 male Lyplal1+/+, n=9 male Lyplal1tm1a/tm1a, n=9 female Lyplal1+/+ and n=7 female Lyplal1tm1a/tm1a).
Changes in *Lyplal1*tm1a/tm1a mice, with small alterations in genes unrelated to metabolism, suggesting that *Lyplal1* is dispensable for normal fat deposition and metabolic control in mice, despite its evolutionary conservation.

Although the evidence implicating the *LYPLAL1* locus in regulation of fat distribution and metabolism was compelling, it is important to remember that the SNPs identified by GWAS are not within *LYPLAL1* itself, and the underlying causal gene or effector...
Mice underwent standardized phenotyping using a modified version of the Sanger Mouse Genetics Project pipeline detailed previously (White et al., 2013), using breeder’s chow (LabDiet 5021, 21% kcal as fat; LabDiet, London, UK) instead of a HFD. *Lyplal1* data from the MGP pipeline and others is available online: http://www.mousephenotype.org/data/genes/MGI:2385115.

HFD studies using Research diets D12451 (45% kcal as fat; Research Diets, New Brunswick, NJ, USA) were performed in three batches, with 11 males and 10 females of each genotype switched from breeder’s chow to HFD at 6 weeks of age. Genotypes and sexes were mixed throughout the batches. Body composition was measured under anaesthesia with ketamine (Ketaject®; Fort Dodge Animal Health, Overland Park, KS, USA) and xylazine (Rompun®; Bayer Animal Health, Leverkusen, Germany) using a PIXImus densitometer (GE Lunar, Madison, WI, USA). Nose-to-tailbase length was measured using a ruler with 1 mm graduations prior to dual-energy X-ray absorptiometry (DEXA). This was performed at 12 (100 mg kg$^{-1}$ ketamine and 10 mg kg$^{-1}$ xylazine) and 22 weeks of age (90 mg kg$^{-1}$ ketamine and 9 mg kg$^{-1}$ xylazine). Quality control was performed using a calibrated phantom before imaging. Anaesthesia was reversed by intraperitoneal injection of atipamezole (1 mg kg$^{-1}$ Antisedan; Orion Pharma, Espoo, Finland).

An intraperitoneal insulin tolerance test was performed on 16-week-old mice after a 6 h fast (08.00-14.00 h), using 0.6 U kg$^{-1}$ Actrapid insulin (Novo Nordisk, Bagsvaerd, Denmark). An intraperitoneal glucose tolerance test was performed on 20-week-old mice after an overnight fast (from 17.00 h, typically 16 h duration), using 2 g kg$^{-1}$ glucose. Mice were individually housed, and ~0.5 mm of the tail tip was removed with a scalpel blade and a fasting blood sample directly taken (Accu-chek Aviva; Roche, Indianapolis, IN, USA). After intraperitoneal injection, further blood samples were taken at 15, 30, 60 and 120 min post-injection. Area under the curve (AUC) was calculated using GraphPad Prism.

Mice were individually housed for 48 h in indirect calorimetry cages (LabMaster system; TSE-systems, Bad Homburg, Germany) at 24 weeks of age. Genotypes and sexes were mixed throughout the batches. Body composition was measured under anaesthesia with ketamine (Ketaject®; Fort Dodge Animal Health, Overland Park, KS, USA) and xylazine (Rompun®; Bayer Animal Health, Leverkusen, Germany) using a PIXImus densitometer (GE Lunar, Madison, WI, USA). Nose-to-tailbase length was measured using a ruler with 1 mm graduations prior to dual-energy X-ray absorptiometry (DEXA). This was performed at 12 (100 mg kg$^{-1}$ ketamine and 10 mg kg$^{-1}$ xylazine) and 22 weeks of age (90 mg kg$^{-1}$ ketamine and 9 mg kg$^{-1}$ xylazine). Quality control was performed using a calibrated phantom before imaging. Anaesthesia was reversed by intraperitoneal injection of atipamezole (1 mg kg$^{-1}$ Antisedan; Orion Pharma, Espoo, Finland).

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Mice were individually housed for 48 h in indirect calorimetry cages (LabMaster system; TSE-systems, Bad Homburg, Germany) at 24 weeks of age. A final blood glucose reading was collected from 26-week-old mice after a 4 h fast, followed by anaesthesia (100 mg kg$^{-1}$ ketamine and 10 mg kg$^{-1}$ xylazine) and culling, with retro-orbital blood and multiple tissue samples collected.

### Ex vivo analysis

Heparinized whole-blood samples were centrifuged at 5000 g for 10 min at 4°C, and the separated plasma was analysed using an Olympus AU400 (Olympus, Tokyo, Japan). Insulin concentrations were determined by ELISA, according to the manufacturer’s instructions (Millipore, Billerica, MA, USA).
Protein extracts were generated from frozen tissue samples by homogenization in tissue protein extraction reagent (TPER) buffer supplemented with Halt protease and phosphatase inhibitor cocktail (both from Thermo Scientific, Rockford, IL, USA), using an Omni TH Tissue homogenizer (Omni International, NW Kennesaw, GA, USA). Western blotting of tissue protein extracts was performed using standard protocols, using the blocking solutions (in TBS with 0.1% Tween 20) and the following antibodies: rabbit anti-Lyplal1 (Proteintech Group, Rosemont, IL, USA; 1:1000), mouse anti-GAPDH (Abcam, Cambridge, UK; 1:5000), goat anti-rabbit (Bio-Rad, Hercules, CA, USA; 1:1000) or goat anti-mouse (Bio-Rad; 1:1000). The blocking solutions were 3% [w/v] milk (for rabbit anti-Lyplal1 and goat anti-rabbit) or 3% [w/v] BSA (for mouse anti-GAPDH and goat anti-mouse). The blots were visualized using Amersham ECL reagents (GE Healthcare, Chicago, IL, USA) and developed using a Xorgraph Imaging Systems Compact X4. Paraffin sections (5 µm thickness) of scWAT were stained with Haematoxylin and Eosin (H&E) and Haematoxylin by conventional methods. Images were collected using a Leica stereomicroscope and a Hamamatsu slide scanner (Hamamatsu, Japan). Adipocyte CSA analysis was calculated from one section of 1.8 mm Leica stereomicroscope and a Hamamatsu slide scanner (Hamamatsu, Japan). Cryosections (10 µm thick) of liver were stained with H&E or Oil Red O and developed using a Xorgraph Imaging Systems Compact X4. Cryosections (10 µm thick) of liver were stained with H&E or Oil Red O and developed using a Xorgraph Imaging Systems Compact X4.

RNA extraction and qPCR
RNA was extracted from snap-frozen mouse tissue using the Qiagen RNeasy plus Universal kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). RNA concentration and purity were evaluated by NanoDrop (Thermo Scientific, Wilmington, DE, USA). RNA integrity was further assessed using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). cdNA synthesis was performed using between 500 ng and 1 µg RNA, random primers and Superscript II reverse transcriptase (Life Technologies, Carlsbad, CA, USA). pCR was performed using Sybr Green (Applied Biosystems, Foster City, CA, USA) and run on an AB7500 qPCR machine (Applied Biosystems). The following primers were used: for 18s, forward 5′-GTAACCGTGGACCCCATTC-3′ and reverse 5′-CCATCCA-ATGGTATAGCCG-3′; Gapdh, forward 5′-TGGTTCACACCCATCACA-AACA-3′ and reverse 5′-GGTGAAGGTCGGTGTGAACGG-3′; for Lyplal1, forward 5′-CACCGTCTAGTCATCTGCG-3′ and reverse 5′-AGGAGGGCTGGTGATAATG-3′; for Rpl32, forward 5′-GGCAGATCTGTATCCCAA-3′ and reverse 5′-GAGGTGCTGCTCTTCTTCTAC-3′. Relative expression for Lyplal1 was calculated using the ΔΔCt method, relative to the cubic mean of three reference genes (Livak and Schmittgen, 2001).

RNaseq
RNaseq was performed on five samples per sex and genotype from gastrocnemius, liver, scWAT and vWAT. Libraries were prepared using the Illumina TruSeq Stranded mRNA Library Preparation Kit with 10 PCR cycles (Illumina, San Diego, CA, USA). Pooling of 20 samples (one pool per organ) were run on three lanes each with 75-bp paired end runs on an Illumina HiSeq 2000 with v4 chemistry, with each pool run on three lanes. Reads were aligned to the NCBI m38 version of the mouse genome, data from multiple lanes combined using Samtools v1.3, mapped reads counted using Feature Counts with duplicates retained and differential expression analysis used DESeq2 (Love et al., 2014). Outliers that did not cluster with the appropriate tissue on the principal components analysis plot were removed. The data were visualized using Integrative Genomics Viewer to confirm the location of any remaining reads in knockout samples (Robinson et al., 2011). RNaseq data are available through the European Nucleotide Archive (ENA), study number PRJEB14194 (https://www.ebi.ac.uk/ena/data/view/PRJEB14194).

Statistical analysis
Unless otherwise stated, statistical analysis was performed as described by Karp et al. (2012), using PhenStat version 2.3.2, using the mixed model framework (Kurbatova et al., 2015). Multiple testing was managed by controlling the family-wise error rate to 5% using the Holm method (Holm, 1979).

\[ Y_{\text{diet}} + \text{Sex} + \text{Sex} \times \text{Sex} + 1 \times \text{Batch} \] (1)

For indirect calorimetry, data analysis was performed in R, using linear model analysis, and correcting for body weight when assessing energy expenditure, O₂ uptake and CO₂ output, and correcting for change in body weight when assessing food intake and respiratory exchange ratio. Interaction terms were checked and were not significant; therefore, these were excluded from the model.

\[ \text{Model} < -\text{lm} (\text{Variable} = 1 + \text{Genotype} + \text{Sex} + \text{Weight} + \text{Batch}) \] (2)

Acknowledgements
The authors thank staff at the Research Support Facility (Sanger Institute) for their excellent care of the mice and both Ed Ryder and members of the genotyping team and Richard Rance and the staff of the WTSI DNA Pipelines Bespoke Sequencing team for their contribution to this work. We would also like to thank Felicity Payne for help with the RNAseq analysis, Rachel Moore for help with the indirect calorimetry analysis, Carl Shannon for help with insulin tolerance tests and advice on mouse welfare, Yvette Hooks for help with histology, Chris Isherwood for help with blood plasma analysis, Lauren Antony and Maks Sumowski for help with insulin and glucose tolerance testing and David Lafont and Mark Sanderson for help with terminal tissue collections. We also thank David Savage for helpful discussions.

Competing interests
The authors declare no competing or financial interests.

Author contributions

Funding
This work was supported by the Wellcome Trust (WT098051 to I.B.).

Data availability
RNAseq data are available through the European Nucleotide Archive (ENA), study number PRJEB14194 (https://www.ebi.ac.uk/ena/data/view/PRJEB14194).

Mouse primary data available at www.mousephenotype.org/data/genes/MGI:2385115.

Supplementary information
Supplementary information available online at http://dmm.biologists.org/lookup/doi/10.1242/dmm.031864.supplemental.

This article has an associated First Person interview with the first author of the paper available online at http://dmm.biologists.org/lookup/doi/10.1242/dmm.031864.supplemental.

References


Figure S1 – Figure demonstrates the conservation of LyplalI across many species. Taken from Ensembl (www.ensembl.org).
Figure S2 – RNAseq results demonstrate that there are only a few detectable reads in exon 1 of Lyplal1. Representative vWAT samples shown, upper two panels show wildtype samples and lower two panels show Lyplal1<sup>tm1a</sup>/<sup>tm1a</sup> samples. Figure was generated using IGV.
Figure S3 – Lyplal1 is knocked out at the protein level. Shown are the original Western blots. Blue marks on the left hand side correspond to Bio-Rad Dual Color Precision Plus Protein markers (250, 150, 100, 75, 50, 37, 25, 20, 15 kDa). Red arrows indicate the band for Lyplal1/GAPDH.
Figure S4—Lyplal1 knockout does not alter body composition in Lyplal1 knockout mice fed high fat diet from 6 weeks of age.

A & B: Nose to tail base length was unaltered in 14-week-old (A) and 24-week-old (B) knockout mice.

C-H: Lean mass (C), fat mass (D) and fat percentage (E), bone mineral density (BMD, F), bone mineral content (BMC, G) and bone area (H) were unaltered in 14-week-old knockout mice, measured by DEXA.

I-J: BMD (I), BMC (J) and bone area (K) were unaltered in 24-week old knockout mice, measured by DEXA.

Black triangles = male Lyplal11/+, white triangles = male Lyplal1tm1a/tm1a, black circles = female Lyplal11/+, white circles = female Lyplal1tm1a/tm1a. Data are presented as means ± s.d. (n=11 males, 9 females per genotype A-H, n=10 males per genotype, n=9 female Lyplal11/+, n=8 female Lyplal1tm1a/tm1a I-K).
Figure S5 – Lypla1 knockout does not alter distribution of adipocyte CSA. Relative adipocyte cross sectional area (CSA) was determined using ImageJ analysis of scWAT sections stained with H&E, and grouped into bins of 1000 µm². Light blue = male Lypla1¹/¹, dark blue = male Lypla1tm1a/tm1a, light purple = female Lypla1¹/¹, dark purple = female Lypla1tm1a/tm1a. Data are presented as means ± s.d. (n=9 male Lypla1¹/¹, n=8 male Lypla1tm1a/tm1a, n=7 female Lypla1¹/¹, n=8 female Lypla1tm1a/tm1a).
Figure S6 – Fasting blood glucose was unaltered in Lyplal1 knockout mice after different length fasts and at different ages.

a: 22-week-old mice were fasted for 16 h overnight before blood glucose measurement. b: 18-week-old mice were fasted for 6 h before blood glucose measurement. c: 28-week-old mice were fasted for 4 h before blood glucose measurement.

Black triangles = male Lyplal1+/+, white triangles = male Lyplal1tm1a/tm1a, black circles = female Lyplal1+/+, white circles = female Lyplal1tm1a/tm1a. Data are presented as means ± s.d., mixed model analysis performed using PhenStat (A: n=6 male Lyplal1+/+, n=6 male Lyplal1tm1a/tm1a, n=6 female Lyplal1+/+, n=5 female Lyplal1tm1a/tm1a; B: n=10 male Lyplal1+/+, n=11 male Lyplal1tm1a/tm1a, n=10 female Lyplal1+/+, n=8 female Lyplal1tm1a/tm1a; C: n=10 male Lyplal1+/+, n=10 male Lyplal1tm1a/tm1a, n=9 female Lyplal1+/+, n=8 female Lyplal1tm1a/tm1a).
**Figure S7** – Lyplal1 knockout does not cause large changes during 48 h indirect calorimetry at 26 weeks of age. Shaded areas show the periods of dark (1930-0730).

A & B: Food intake was unaltered by genotype or sex. C & D: There was a significant effect of genotype on VO2 (p=0.038 for genotype, linear model). E & F: VCO2 was unaltered by genotype or sex. G-I: Raw data graphs for X activity, Y activity, RER and energy expenditure.

Black or light blue triangles = male Lyplal1\textsuperscript{+/+}, white or dark blue triangles = male Lyplal1\textsuperscript{tm1a/tm1a}, black or light purple circles = female Lyplal1\textsuperscript{+/+}, white or dark purple circles = female Lyplal1\textsuperscript{tm1a/tm1a}. Data are presented as means ± s.d. (A-B: n=9 male Lyplal1\textsuperscript{+/+}, n=8 male Lyplal1\textsuperscript{tm1a/tm1a}, n=6 female Lyplal1\textsuperscript{+/+}, n=5 female Lyplal1\textsuperscript{tm1a/tm1a}; C-F: n=9 male Lyplal1\textsuperscript{+/+}, n=8 male Lyplal1\textsuperscript{tm1a/tm1a}, n=6 female Lyplal1\textsuperscript{+/+}, n=6 female Lyplal1\textsuperscript{tm1a/tm1a}; G-I: n=9 male Lyplal1\textsuperscript{+/+}, n=8 male Lyplal1\textsuperscript{tm1a/tm1a}, n=6 female Lyplal1\textsuperscript{+/+}, n=7 female Lyplal1\textsuperscript{tm1a/tm1a}).

**Table S1.** No changes in mass of the listed organs in 28-week-old knockout mice. Mixed model analysis performed using PhenStat (n=10 male Lyplal1\textsuperscript{+/+}, n=10 male Lyplal1\textsuperscript{tm1a/tm1a}, n=9 female Lyplal1\textsuperscript{+/+}, n=8 female Lyplal1\textsuperscript{tm1a/tm1a}).

<table>
<thead>
<tr>
<th>Organ weight (g)</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Lyplal1\textsuperscript{+/+}</td>
<td>Lyplal1\textsuperscript{tm1a/tm1a}</td>
<td>Lyplal1\textsuperscript{+/+}</td>
<td>Lyplal1\textsuperscript{tm1a/tm1a}</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>0.151 ± 0.010</td>
<td>0.151 ± 0.009</td>
<td>0.126 ± 0.006</td>
<td>0.141 ± 0.015</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>0.076 ± 0.012</td>
<td>0.076 ± 0.013</td>
<td>0.059 ± 0.009</td>
<td>0.059 ± 0.009</td>
</tr>
<tr>
<td>Heart</td>
<td>0.157 ± 0.013</td>
<td>0.156 ± 0.020</td>
<td>0.128 ± 0.019</td>
<td>0.140 ± 0.012</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.209 ± 0.021</td>
<td>0.209 ± 0.016</td>
<td>0.159 ± 0.014</td>
<td>0.158 ± 0.014</td>
</tr>
<tr>
<td>Liver</td>
<td>2.330 ± 0.706</td>
<td>2.405 ± 0.660</td>
<td>1.504 ± 0.262</td>
<td>1.529 ± 0.244</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.098 ± 0.023</td>
<td>0.099 ± 0.016</td>
<td>0.104 ± 0.017</td>
<td>0.106 ± 0.017</td>
</tr>
</tbody>
</table>
First person – Rachel Watson

How would you explain the main findings of your paper to non-scientific family and friends?

Studies have been performed to identify factors which increase risk of certain diseases such as obesity, diabetes and other metabolic problems. Some of these studies, called genome-wide association studies (GWAS), identify changes in the genetic code that impact on disease risk. These changes are often found in regions between genes, meaning that they are in regions that do not code for the building blocks in our body (proteins). How these changes can influence risk of disease can therefore be difficult to understand, and it may be that they affect how much of a particular protein is made, where the gene coding for that protein is nearby, but often this cannot be mathematically discerned from the data. Several studies had shown that DNA variations that occurred close to the LYPLAL1 gene were associated with metabolic disease. As little was known about how this gene worked, we studied mice without any Lyplal1 to see how this affected them. The mice were normal in all the metabolic tests we performed, which indicates that LYPLAL1 is not important in controlling metabolism, and that this identified change may be acting through a different gene.

What are the potential implications of these results for your field of research?

These results suggest that a different gene other than LYPL1 may be responsible for the signals identified by GWAS, which has up to now been attributed to LYPLAL1. The results also highlight the caution that must be taken when interpreting GWAS results. This has also been well documented in the case of the identification of the causal gene around the FTO locus.

What are the main advantages and drawbacks of the model system you have used as it relates to the disease you are investigating?

Mice are an excellent model system for investigating metabolic disease. The increase in the use of human iPSCs has been very valuable to research; however, these cannot recreate the complexity of multicellular organism. Studies in mice allow us to investigate the effect on a living organism, which is particularly relevant for metabolic disease where phenotypes are affected by multiple organs, and where parameters such as food intake and exercise are important. Although it was not used in our study, the emergence of the CRISPR technology also allows much more rapid generation of mouse mutant models, where speed was previously a major drawback of this system.

What has surprised you the most while conducting your research?

Given the number of papers linking LYPLAL1 to metabolic disease phenotypes, it was surprising to see no phenotype. However, as discussed above and in the paper, GWAS loci normally map to a non-coding region, and these are then suggested to be affecting the phenotype via the nearest gene, which is not always going to be
correct. This indicates the importance of functional follow-up of GWAS loci to confirm the effector gene.

"...negative results are extremely important, as they can challenge widely held assumptions."

Describe what you think is the most significant challenge impacting your research at this time and how will this be addressed over the next 10 years?

Our paper demonstrates the absence of a phenotype in Lyplal1 knockout mice. A major challenge affecting model organism research currently is the reluctance of many journals to publish such ‘negative’ results, and of researchers themselves to present them at conferences and to invest the time and effort to complete such studies in a rigorous manner once it appears likely that no phenotype is going to be discovered. However, as discussed in the paper, these negative results are extremely important, as they can challenge widely held assumptions, such as the assumption that LYPLAL1 was the gene through which the GWAS loci were acting. In addition to the scientific value of negative results, it also has the consequence of causing a large waste in resources, as multiple different researchers generate similar models which never reach publication.

Reference